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(54) Title: DRY HEMOSTATIC COMPOSITIONS AND METHODS FOR THEIR PREPARATION

(57) Abstract: Dry cross-linked gelatin compositions are prepared that rapidly re-hydrate to produce gelatin hydrogels suitable as hemostatic sealants. Gelatin is cross-linked in the presence of certain re-hydration aids, such as polyethylene glycol, polyvinyl-providone, and dextran, in order to produce a dry cross-linked gelatin powder. The use of the re-hydration aids has been found to substantially increase the re-hydration rate in the presence of an aqueous re-hydration medium, typically thrombin-containing saline.



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DRY HEMOSTATIC COMPOSITIONS AND METHODS FOR THEIR PREPARATION

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

The present invention relates generally to collagen and collagen-derived compositions and methods for their preparation. In particular, the present invention relates to a method for producing a dry cross-linked gelatin or other collagen or collagen-derived composition which is capable of absorbing water at an enhanced rate.

10 Fusion Medical Technologies, Inc., assignee of the present application, produces a hemostatic composition under the FloSeal® trade name. The FloSeal® product is available in a package including two syringes. A first syringe is filled with granules of cross-linked bovine gelatin which are pre-hydrated with a buffer solution. The gelatin hydrogel contains about 85% (w/w) water and is in the form of a flowable hydrogel. Immediately
15 prior to use in the operating room, thrombin in aqueous saline is mixed with the gelatin hydrogel. The thrombin is prepared in saline and drawn up in a second syringe, and the syringes are connected together permitting mixing of thrombin and the gelatin.

The resulting mixture of the gelatin hydrogel granules and the thrombin has been found to be a highly effective hemostatic sealant when applied to a bleeding site.
20 Typically, the sealant will be applied through the syringe in which it has been mixed to the bleeding site. Blood will percolate through the resulting bed of hydrogel granules, and the thrombin reacts with fibrinogen in the blood to form a fibrin clot around the gelatin to seal the bleeding site.

Although highly effective, the present FloSeal® product has a limited shelf
25 life. It is believed that the stability of the gelatin is reduced by hydrolysis of the packaged hydrogel. To limit possible hydrolytic degradation, the FloSeal® product is usually shipped in a temperature-protected packaging.

For these reasons, it would be desirable to provide improved hemostatic sealing compositions of the type which combine a collagen, gelatin, or other collagen-derived
30 hydrogel with a thrombin-containing aqueous solution. In particular, it would be desirable to provide such compositions in a form which would be resistant to hydrolytic degradation and which would therefore have a longer shelf life. It would be particularly desirable to provide

improved compositions having both comparable hemostatic activity to the present FloSeal[®] product and longer shelf lives. Such compositions would be most beneficial if they could be rapidly re-hydrated for subsequent use, typically so that they could be extruded through a syringe. At least some of these objectives will be met by the inventions described

hereinafter.

2. Description of the Background Art

The FloSeal[®] product available from Fusion Medical Technologies, Inc., is described in Hood et al., *Efficacy of Topical Hemostat FloSeal[™] in Vascular Surgery*, an Abstract funded by Fusion Medical Technologies, Inc., which was publicly presented in September 1999. Patents covering the FloSeal[®] product include U.S. Patent Nos. 6,063,061 and 6,066,325. A dual syringe system suitable for mixing and delivering a collagen, gelatin, or other collagen-derived component and a thrombin component of the FloSeal[™] product is described in U.S. Patent No. 5,908,054. The complete disclosures of each of these patent references is hereby incorporated by reference.

BRIEF SUMMARY OF THE INVENTION

The present invention provides improved hemostatic sealing compositions, methods for preparing such improved compositions, and kits comprising the improved compositions. The methods and compositions will be particularly useful for providing hemostasis at bleeding sites, including surgical bleeding sites, traumatic bleeding sites and the like. An exemplary use of the compositions may be in sealing the tissue tract above a blood vessel penetration created for vascular catheterization.

The compositions comprise a dry cross-linked gelatin powder which has been prepared to re-hydrate rapidly. The gelatin powder preferably comprises relatively large particles, also referred to as fragments or sub-units, as described in U.S. Patent Nos. 6,063,061 and 6,066,325, the full disclosures of which have previously been incorporated by reference. A preferred particle size will be the range from 150 μm to 750 μm , but particle sizes outside of this preferred range may find use in many circumstances. The dry compositions will also display a significant "equilibrium swell" when exposed to an aqueous re-hydrating medium. Preferably, the swell will be in the range from 400% to 1000%, but may fall outside of this range as set forth in the above-referenced patents. "Equilibrium swell" may be determined by subtracting the dry weight of the gelatin hydrogel powder from its weight when fully hydrated and thus fully swelled. The difference is then divided by the dry weight and multiplied by 100 to give the measure of swelling. The dry

weight should be measured after exposure of the material to an elevated temperature for a time sufficient to remove substantially all residual moisture, e.g., two hours at 120°C. The equilibrium hydration of the material can be achieved by immersing the dry material in a suitable re-hydrating medium, such as aqueous saline, for a time period sufficient for the water content to become constant, typically for from 18 to 24 hours at room temperature.

The dry cross-linked gelatin powders of present invention will usually have some residual moisture, but will be sufficiently dry to achieve the desired stability and extended shelf life. Typically, the dry compositions will have a moisture content below 20% by weight (w/w) or less, preferably having a moisture content in the range from 5% by weight to 15% by weight. To maintain dryness, the compositions will typically be packaged in a manner suitable to prevent moisture incursion, as described in more detail in connection with the kits of the present invention.

In one particular aspect of the present invention, compositions will comprise cross-linked gelatin powders having a moisture content of 20% (w/w) or less, wherein the powder was cross-linked in the presence of a re-hydration aid so that the powder has an aqueous re-hydration rate which is at least 5% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid. The "re-hydration rate" is defined herein to mean the quantity of an aqueous solution, typically 0.9% (w/w) saline, that is absorbed by a gram of the powder (dry weight basis) within thirty seconds, expressed as gm/gm. Particular techniques for measuring this rate are described in the Experimental section hereinafter. Preferred compositions of the present invention will have a re-hydration rate of at least 3 gm/gm, preferably at least 3.5 gm/gm, and often 3.75 gm/gm or higher. Re-hydration rates of similar powders prepared without the re-hydration aids are typically below three, and a percentage increase in re-hydration rate will usually be at least 5%, preferably being at least 10%, and more preferably being at least 25% or higher.

The dry cross-linked gelatin powders of the present invention having improved re-hydration rates are preferably obtained by preparing the powders in the presence of certain re-hydration aids. Such re-hydration aids will be present during the preparation of the powders, but will usually be removed from the final products. For example, re-hydration aids which are present at about 20% of the total solids content, will typically be reduced to below 1% in the final product, often below 0.5% by weight. Exemplary re-hydration aids include polyethylene glycol (PEG), preferably having a molecular weight of about 1000; polyvinylpyrrolidone (PVP), preferably having an average molecular weight of about 50,000; and dextran, typically having an average molecular weight of about 40,000. It is preferred to

employ at least two of these re-hydration aids when preparing the compositions of the present invention, and more particularly preferred to employ all three.

The methods of the present invention thus comprise providing an aqueous solution of a non-cross-linked gelatin combined with a re-hydration aid. The non-cross-linked gelatin will typically be present in an aqueous solution at from 5% (w/w) to 15% (w/w) and the re-hydration aids will be typically present from 5% to 30% (w/w) based on the weight of gelatin in the aqueous solution. Preferably, the re-hydration aid comprises PEG at from 2.5% to 20% (w/w) based on the weight of the gelatin, PVP at from 1.25% to 20% (w/w), and dextran at from 1.25% to 20% (w/w).

The non-cross-linked gelatin together with the re-hydration aid is then cross-linked in any manner suitable to form the hydrogel. For example, polymeric molecules may be cross-linked using bi- or poly-functional cross-linking agents which covalently attach to two or more polymer molecules chains. Exemplary bifunctional cross-linking agents include aldehydes, epoxies, succinimides, carbodiimides, maleimides, azides, carbonates, isocyanates, divinyl sulfone, alcohols, amines, imidates, anhydrides, halides, silanes, diazoacetate, aziridines, and the like. Alternatively, cross-linking may be achieved by using oxidizers and other agents, such as periodates, which activate side-chains or moieties on the polymer so that they may react with other side-chains or moieties to form the cross-linking bonds. An additional method of cross-linking comprises exposing the polymers to radiation, such as gamma radiation, to activate the polymer chains to permit cross-linking reactions. Dehydrothermal cross-linking methods may also be suitable. Preferred methods for cross-linking gelatin molecules are described below.

Exemplary methods for producing cross-linked gelatins are as follows.

Gelatin is obtained and suspended in an aqueous solution to form a non-cross-linked hydrogel, typically having a solids content from 1% to 70% by weight, usually from 3% to 10% by weight. The gelatin is cross-linked, typically by exposure to either glutaraldehyde (e.g., 0.01% to 0.05% w/w, overnight at 0 C to 15 C in aqueous buffer), sodium periodate (e.g., 0.05 M, held at 0°C to 15°C for 48 hours) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ("EDC") (e.g., 0.5% to 1.5% w/w overnight at room temperature), or by exposure to about 0.3 to 3 megarads of gamma or electron beam radiation. Alternatively, gelatin particles can be suspended in an alcohol, preferably methyl alcohol or ethyl alcohol, at a solids content of 1% to 70% by weight, usually 3% to 10% by weight, and cross-linked by exposure to a cross-linking agent, typically glutaraldehyde (e.g., 0.01% to 0.1% w/w, overnight at room temperature). In the case of aldehydes, the pH should be held from about 6

to 11, preferably from 7 to 10. When cross-linking with glutaraldehyde, the cross-links are formed via Schiff bases which may be stabilized by subsequent reduction, e.g., by treatment with sodium borohydride. After cross-linking, the resulting granules may be washed in water and optionally rinsed in an alcohol, and dried. The resulting dry powders may then be loaded
5 into the applicators of the present invention, as described in more detail hereinafter.

After cross-linking, at least 50% (w/w) of the re-hydration aid will be removed from the resulting hydrogel. Usually, the re-hydration aid is removed by filtration of the hydrogel followed by washing of the resulting filter cake. Such filtration/washing steps can be repeated one or more additional times in order to clean the product to a desired level and
10 to remove at least 50% of the re-hydration aid, preferably removing at least 90% (w/w) of the re-hydration aid originally present.

After filtration, the gelatin is dried, typically by drying the final filter cake which was produced. The dried filter cake may then be broken up or ground to produce the cross-linked powder having a particle size in the desired ranges set forth above.

Kits according to the present invention will comprise a first container holding the dry cross-linked gelatin powder of the present invention, as described above. The kits will further comprise a second container holding an aqueous re-hydration medium, typically a saline or other aqueous solution comprising thrombin which is intended to be mixed with the gelatin as the gelatin is re-hydrated. The containers can be in any form, but will preferably be
15 in the form of syringes which permit mixing of the dry gelatin with the re-hydration medium.
20

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a kit constructed in accordance with the principles of the present invention.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The following examples are offered by way of illustration, not by way of
25 limitation.

Example 1: Preparation of gelatin powder

Strips of bovine corium were suspended in a sodium hydroxide solution of concentration 1 M to 2 M for 1 hr at room temperature, neutralized with phosphoric acid, and
30 rinsed. The treated strips were then resuspended in deionized water, adjusted to pH 7-8, and heated to 70°C. A homogenizer was used to further reduce the size of the strips. After 1 hr at 70°C, the corium was largely solubilized to gelatin. The amount of corium was chosen so

that the solids content of the resulting gelatin solution was approximately 3-10% (w/w), typically 7-10%. The solution was cast as thin layers onto Teflon® coated metal trays, dried, and ground to form gelatin powder.

Example 2: Preparation of “modified gelatin powder”

5 Re-hydration aids (Table 1) were dissolved in 500 mL of 50°C de-ionized water and then an amount of bovine derived gelatin powder, prepared as in Example 1, was added to the solution. The final concentration of gelatin in solution was chosen to be approximately 8% (w/w, bulk gelatin powder basis), and the total amount of re-hydration aids in the solution was chosen as in Examples 9-44 (Tables 1 and 2). After the gelatin had
10 dissolved, the solution was poured into Teflon® coated metal trays and dried. The dried gelatin sheet is ground to form “modified gelatin powder”.

 Alternatively, strips of bovine corium were suspended in a sodium hydroxide solution of concentration 1 M to 2 M for 1 hr at room temperature, neutralized with phosphoric acid, and rinsed. The treated strips were then resuspended in deionized water,
15 adjusted to pH 7-8, and heated to 70°C. A homogenizer was used to further reduce the size of the strips. After 1 hr at 70°C, the corium was largely solubilized to gelatin. The amount of corium was chosen so that the solids content of the resulting gelatin solution was approximately 3-10% (w/w), typically 7-10%. Amounts of re-hydration aids were chosen as in Examples 9-44 (Tables 1 and 2) and were then added to the gelatin solution, either in solid
20 form or dissolved in a small volume of water. The solution was cast into thin layers onto Teflon® coated metal trays, dried, and ground to form “modified gelatin powder”. Examples of several formulations for modified gelatin are given in Tables 1 and 2.

Example 3: Preparation of cross-linked gelatin powder from “modified gelatin powder”

25 600 mL of 0.2 M phosphate buffer (pH 9.2 ± 0.2) was cooled to a temperature below 12°C. 0.32 mL of glutaraldehyde (25%) was added to the buffer solution and then 20 g of modified gelatin powder was added, resulting in a glutaraldehyde concentration of 4000 ppm (glutaraldehyde to modified gelatin, bulk weight basis). The gelatin was suspended in the glutaraldehyde solution with a stir bar. The pH of each suspensions was adjusted to a
30 range of 9.2 ± 0.2 and then maintained at a temperature of 9 to 12°C and pH of 9.2 ± 0.2 over 19 hours.

 The suspension was filtered and the filter cake was washed with de-ionized water three times by completely covering the filter cake with de-ionized water and then

allowing the vacuum to draw the rinse water through the cake. The filter cake was left in the funnel during each rinse.

0.2 g of NaBH₄ was dissolved in 600 mL 25 mM phosphate buffer, pH 7.4
0.2, in a beaker. The above filter cake was suspended in the NaBH₄ solution at room
5 temperature (about 22°C) for 3 hours, then filtered to remove the liquid.

The filter cake was next suspended in 600 mL of buffer solution at room
temperature (about 22°C) for 30 minutes and filtered again. The buffer was composed of
sodium phosphate (dibasic anhydrous and monobasic monohydrate) and sodium ascorbate.
The above procedure was repeated twice to ensure that the appropriate ratio of salts to gelatin
10 were present to form the desired buffer composition upon reconstitution. The filter cake was
dried, then ground with a Waring Blender, resulting in "cross-linked gelatin powder".

This method was also used to prepare cross-linked gelatin powder from
unmodified gelatin powder; that is, gelatin to which no re-hydration aids were added during
its preparation.

15 Example 4: Preparation of irradiated product from cross-linked gelatin
powder

About 800 mg (bulk weight) of the cross-linked gelatin powder, prepared as in
Example 2, were put into each of several 5 cc syringes. The syringes containing powder
were sterilized with gamma irradiation at ambient temperature.

20 Example 5: Use of product as a hemostatic agent

A syringe of product containing approximately 0.8 g of irradiated cross-linked
gelatin powder was prepared from modified gelatin powder. The modified gelatin powder
was prepared as in Example 2. The modified gelatin was further cross-linked and irradiated
as in Examples 3 and 4. The product was mixed with 4 mL of a saline solution containing
25 about 1000 Units of bovine thrombin per milliliter. Mixing was achieved by passage back
and forth between two syringes connected with a female-female straight-through Luer
connector. The powder in the syringe was hydrated as it mixed with the thrombin solution,
forming granules of hydrogel.

A square lesion, approximately 1 cm x 1 cm x 0.2 cm deep, was created on the
30 liver of a farm-grade pig. The pig had been anticoagulated with heparin so that its activated
clotting time (ACT) was three to five times its baseline value, and the lesion bled freely prior
to treatment. After about 30 seconds from the start of mixing, approximately 2 mL of the
hydrated powder was extruded from the syringe onto the lesion and held in place with
compression for two minutes. After compression was removed, the treated lesion was

observed for bleeding at 3 min, 10 min, and 50 min after application. No bleeding was seen from the treated lesion at the 3 min and 10 min observation. After the 10 min observation, the treated lesion was irrigated with saline solution. While excess material was removed, no re-bleeding was observed. At 50 min after application, the lesion was observed again and no bleeding was seen.

Example 6: Determination of re-hydration rate of a powder

The “re-hydration rate” of a powder was measured as follows. The powder, packed in a 5 cc syringe, was mixed with a syringe containing a volume of aqueous solution by passage between the two syringes connected with a Luer fitting for 30 seconds. The volume of aqueous solution was chosen to be in excess of what could be expected to be absorbed in 30 seconds. Typically, 0.8 g (bulk weight) of powder was mixed with 3 mL of 0.9% sodium chloride solution. The resulting mixture was then immediately filtered to remove any unabsorbed liquid. The wet filtered material was weighed, then dried in a 120°C oven for two hours and re-weighed. This measurement gave the total amount of water removed from the wet material and the weight of the dry powder. The amount of water that had been absorbed by the powder was then calculated after a small correction is made for the residual moisture that had been present in the powder originally. The “re-hydration rate” was given as the mass of saline solution absorbed per gram dry weight of powder in that 30 second interval.

In the calculation below, the fraction solids of the bulk powder (“S”) was measured independently by drying the bulk powder at 120°C for 2 hr and weighing the powder before and after drying. The value of S is given by the following:

$$S = \frac{\text{weight after drying at } 120^{\circ} \text{ C, 2hr}}{\text{weight before drying}}$$

Re-hydration rate calculation:

A: initial weight of the pan and filter paper

B: weight of the pan, filter paper and hydrated powder

C: weight of the pan, filter paper and sample after drying in oven

S: fraction solids of the bulk powder originally in syringe

M: grams of saline absorbed per gram of powder (dry weight) during mixing (“absorption rate”)

$$M = \frac{(B - A) - (C - A) / S}{(C - A)}$$

Example 7: Re-hydration rate and physical property determination for several batches of powder product

Tables 1 and 2 depict the results of re-hydration rate measurements performed on one to for several batches of powder product (Examples 9-23). These were made using methods as per Examples 1, 2, 3, and 4. Except for Examples 9 and 17, these were prepared from modified gelatins that were made with various proportions of gelatin and the following re-hydration aids: polyethylene glycol (PEG), average molecular weight 1000; polyvinylpyrrolidone (PVP), "k-30" designation, corresponding to an average molecular weight of about 50,000; and dextran, average molecular weight 40,000.

It is seen that use of several different combinations of gelatin and re-hydration aids can result in a powder product that absorbs more aqueous saline solution in 30 seconds per gram of powder than powder product made from gelatin to which no re-hydration aids have been added. It is also seen that the combination of gelatin, PEG, PVP and dextran at a bulk weight ratio of 80:10:5:5 in the modified gelatin (Example 10) produces a powder product that absorbs about 33% more saline solution per gram in 30 seconds than powder product made from unmodified gelatin.

Table 1 also gives values for other physical properties determined for the powder product lots. "Percent solids" was determined by weighing the powder before and after drying at 120°C for two hours to drive off residual moisture. "DSC peak temperature" refers to the temperature at which a peak is exhibited in a thermogram of a differential scanning calorimetry measurement conducted from 1°C to 70°C. "Equilibrium swell" was determined by suspending the powder in an excess of saline solution for at least 18 hr at room temperature. The hydrated powder was weighed to determine its "equilibrium wet weight" and dried at 120°C for two hours and re-weighed to determine its "dry weight". Equilibrium swell is given as

$$\text{Equilibrium swell (\%)} = 100\% \times \frac{\text{equilibrium wet weight} - \text{dry weight}}{\text{dry weight}}$$

Values for "mean particle size" were measured by light scattering in a Coulter LS particle size analyzer.

From the data presented in Table 1, it appears that the appropriate use of re-hydration aids can change the re-hydration rate of the powder product without significantly changing other physical properties.

Example 8: Measurement of polyethylene glycol, polyvinylpyrrolidone, and dextran levels in modified gelatin powder and in cross-linked powder

Approximately 50 mg modified gelatin or 250 mg cross-linked irradiated powder product were suspended in 10 mL of deionized water and heated for 3 hr at 65°C.

5 The samples were then centrifuged at 15 minutes at 2000 rpm. The resulting supernatant was filtered through a 0.45 μ m Gelman Acrodisc filter, the first mL being discarded. The resulting sample was then assayed by three different high performance liquid chromatography (HPLC) methods to quantitate the polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and dextran in the sample. For PEG, 100 μ L of the sample was
10 injected onto a Waters Ultrahydrogel 120 column, 7.8 x 300 mm, with guard column and prefilter, using deionized water as the mobile phase. A refractive index detector was used to monitor the effluent. For PVP, 100 μ L of the sample was injected onto a Phenomenex Kingsorb C18 5 μ m column, 4.6 x 150 mm, with guard column and prefilter, using a gradient of methanol and aqueous sodium phosphate as the mobile phase. An ultraviolet absorbance
15 detector was used to monitor the effluent. For dextran, 100 μ L of the sample was injected onto a Waters Ultrahydrogel Linear column, 7.8 x 300 mm, with guard column and prefilter, using 0.1 M sodium phosphate, pH 7 and acetonitrile at a 90:10 ratio as the mobile phase. A refractive index detector was used to monitor the effluent. All columns were heated to 40°C for the analyses. The limit of quantitation was about 0.1% (w/w sample) for PEG and PVP,
20 0.2% (w/w sample) for dextran.

Modified gelatin was prepared as per Example 2. The modified gelatin was analyzed for PEG, PVP and dextran in the manner described above. Results indicated that PEG, PVP, and dextran were present at 16%, 8%, and 3% (w/w bulk) respectively. The modified gelatin was subsequently subjected to cross-linking, sodium borohydride treatment,
25 and rinsing as per Example 3 to form cross-linked modified gelatin powder. When this powder was analyzed for PEG, PVP, and dextran by HPLC in the manner described above, the content of each of the three re-hydration aids was found to be below the limit of quantitation.

Example 9: Powder product made without re-hydration aids

30 Unmodified gelatin—that is, gelatin to which processing aids were not added—was prepared from bovine corium strips as in Example 1 and cross-linked as in Example 3. The cross-linked unmodified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting product were measured as in Examples 6 and 7 and are given in Table 1.

Examples 10-23: Powder product made with re-hydration aids

Batches of modified gelatin were prepared as in Example 2 from gelatin powder or corium strips and from one, two, or three re-hydration aids. Table 1 gives the proportions of bulk gelatin and re-hydration aids used. The modified gelatin was then cross-linked as in Example 3. Except for Example 17, the re-hydration aids used were from the following list: polyethylene glycol (PEG) of an average molecular weight of about 1000; polyvinylpyrrolidone (PVP), "k-30" designation, of an average molecular weight of about 50,000; and dextran, of an average molecular weight of about 40,000. In Example 17, PEG of an average molecular weight of about 400 was used. The cross-linked modified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting powder product from each of these preparations were measured as in Examples 6 and 7 and are given in Table 1. Data given with the formulation for Example 10 is the average and standard deviation of nine batches prepared according to that formulation.

Examples 24-44: Powder product made with various re-hydration aids

Batches of modified gelatin were prepared as in Example 2 from gelatin powder or corium strips and from one of several re-hydration aids. Table 2 gives the identity and concentration of re-hydration aid used in each batch as a ratio of bulk gelatin weight to re-hydration aid and as a percentage of total bulk solute used to prepare the modified gelatin. The modified gelatin was then cross-linked as in Example 3. The cross-linked modified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting powder product from each of these preparations were measured as in Examples 6 and 7 and are given in Table 2. Data for the Example 9 formulation is provided in Table 2 for comparison.

TABLE 1

	Lot	Target bulk weight percent in modified gelatin				Properties of powder product after cross-linking and gamma irradiation (re-hydration aids largely removed)				
		Gelatin (bulk weight)	PEG MW = 1000 D	PVP MW ~ 50000 D	Dextran MW = 40000 D	% solids	DSC peak temp (°C)	Equilibrium swell (%)	Mean particle size (µm)	Re-hydration*
No re-hydration aids added										
Example 9	208-32	100	0	0	0	88.6	41.3	551	440	2.85
Preferred composition (four-way mixture)										
Example 10	avg of 9 lots	80	10	5	5	87.6	42.1	595	423	3.79
	std. deviation of 9 lots					1.0	1.4	43	65	0.15
Three-way mixtures										
Example 11	228-69-1	80	10	10	0	88.1	40.8	667	387	3.51
Example 12	228-69-2	80	10	0	10	88.4	40.6	670	367	3.14
Example 13	228-78	80	0	10	10	86.7	41.1	632	414	3.20
Gelatin-PEG mixtures										
Example 14	212-39-2	94	6	0	0	86.2	44.4	502	372	2.68
Example 15	228-42-3	89	11	0	0	88.6	42.8	594	428	3.16
Example 16	228-42-1	80	20	0	0	88.9	42.4	575	312	3.47
Example 17	214-62-1	89	11**	0	0	87.1	40.7	599	406	3.11
Gelatin-PVP mixtures										
Example 18	228-38-3	94	0	6	0	88.2	42.2	567	399	3.26
Example 19	228-38-2	89	0	11	0	88.3	41.0	605	422	3.44
Example 20	228-38-1	80	0	20	0	88.6	42.4	596	401	3.52
Gelatin-dextran mixtures										
Example 21	228-35-3	94	0	0	6	88.1	40.5	631	395	3.18
Example 22	228-35-2	89	0	0	11	88.3	41.4	598	345	3.03
Example 23	228-35-1	80	0	0	20	88.5	41.9	624	392	3.01
*-Re-hydration rate defined as grams saline absorbed per gram powder product (dry wt) in 30 sec										
**-PEG (MW =400) used instead of MW=1000										

TABLE 2

	Lot	Re-hydration aid			Conc'n of processing aid in	Physical properties				
		type	MW or other designation	bulk gelatin wt: aid		% solids	DSC peak temp (°C)	Equilibrium swell (%)	Mean particle size (µm)	Re-hydration rate*
Example 9	208-32		no re-hydration aids			88.6	41.3	551	440	2.85
Example 24	214-11-1	glycerol	n/a	4	20%	85.5	43.4	483	653	2.19
Example 25	214-11-2	glycerol	n/a	8	11%	86.4	43.4	529	421	2.62
Example 26	214-11-3	glycerol	n/a	16	6%	86.5	43.0	543	398	2.35
Example 27	214-44-1	dextran	148000 D	4	20%	85.5	nr	634	433	2.62
Example 28	214-44-2	dextran	148000 D	8	11%	85.4	nr	607	453	2.57
Example 29	214-44-3	dextran	148000 D	16	6%	85.5	nr	603	527	2.33
Example 30	214-44-4	dextran	148000 D	32	3%	85.7	nr	531	491	2.37
Example 31	228-35-4	dextran	40000 D	32	3%	84.5	41.4	633	380	2.59
Example 32	214-50-1	PVP	k-90	4	20%	85.3	44.0	612	664	2.41
Example 33	214-50-2	PVP	k-90	8	11%	85.6	44.3	538	581	2.71
Example 34	214-50-3	PVP	k-90	16	6%	85.6	44.1	527	593	2.78
Example 35	214-50-4	PVP	k-90	32	3%	86.1	43.0	597	538	2.76
Example 36	214-53-4	PVP	k-30	32	3%	87.3	41.1	580	447	2.72
Example 37	214-59-1	PEG	400	4	20%	86.7	42.0	595	407	2.18
Example 38	214-66-1	PEG	400	6	14%	86.5	40.8	603	501	2.63
Example 39	212-39-1	PEG	400	16	6%	86.2	43.8	513	403	2.11
Example 40	212-39-2	PEG	1000	16	6%	86.2	44.4	502	372	2.68
Example 41	214-59-3	PEG	8000	4	20%	87.4	41.5	548	429	2.87
Example 42	214-66-3	PEG	8000	6	14%	86.9	41.4	581	426	3.80
Example 43	214-62-3	PEG	8000	8	11%	86.8	42.0	631	511	2.78
Example 44	212-39-3	PEG	8000	16	6%	86.4	44.6	546	518	2.72

nr = not reported *Re-hydration rate defined as grams saline absorbed per gram powder product (dry wt) in 30 sec

While the above is a complete description of the preferred embodiments of the invention, various alternatives, modifications, and equivalents may be used. Therefore, the above description should not be taken as limiting the scope of the invention which is defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing a substantially dry cross-linked gelatin powder, said method comprising:
 - providing an aqueous solution comprising linked gelatin combined with at least one re-hydration aid;
 - drying the solution of the gelatin and re-hydration aid to produce solids;
 - grinding the solids to produce a powder;
 - cross-linking the powder;
 - removing at least 50% (w/w) of the re-hydration aid; and
 - drying the cross-linked gelatin to produce a powder having a moisture content below 20% (w/w).
2. A method as in claim 1, wherein the re-hydration aid comprises one, two, or three of the materials selected from the group consisting of polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and dextran.
3. A method as in claims 1 or 2, wherein the re-hydration aid is present at a concentration in the range from 5% to 30% by weight based on the weight of gelatin present in the aqueous solution.
4. A method as in claim 3, wherein the re-hydration aid comprises PEG at from 2.5% to 20% by weight, PVP at from 1.25% to 20% by weight, and dextran at from 1.25% to 20% by weight.
5. A method as in any of the preceeding claims, wherein cross-linking comprises adding a cross-linking agent to the gelatin solution.
6. A method as in claim 5, wherein the cross-linking agent comprises glutaraldehyde.
7. A method as in any of the preceeding claims, wherein removing at least 50% of the re-hydration aid comprises filtering the suspension of cross-linked powder in a solvent followed by filtration gelatin to produce a filter cake and washing the filter cake to remove the re-hydration aid.

8. A method as in claim 7, wherein washing the filter cake removes at least 90% (w/w) of the re-hydration aid originally present in the gelatin.
9. A method as in any of the preceeding claims, wherein drying comprises drying the filter cake after washing, wherein the method further comprises drying the ground filter cake to produce the dry ground gelatin powder.
10. A composition comprising cross-linked gelatin powder having a moisture content of 20% (w/w) or less, wherein the powder was cross-linked in the presence of a re-hydration aid so that the powder has an aqueous re-hydration rate which is at least 5% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid.
11. A composition as in claim 10, wherein the powder has a re-hydration rate which is at least 10% higher and optionally at least 25% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid.
12. A composition as in claims 10 or 11, wherein the re-hydration aid comprises one, two, or three of the materials selected from the group consisting of polyethylene glycol (PEG) polyvinylpyrrolidone (PVP), and dextran.
13. A composition as in any of the preceeding claims, wherein the powder has a mean particle size in the range from 150 μm to 750 μm .
14. A composition as in any of the preceeding claims, wherein the powder has an equilibrium swell in the range from 400% to 1000%.
15. A kit comprising:
 - a first container holding cross-linked gelatin powder any of claims 12-19; and
 - a second container holding an aqueous re-hydration medium.
16. A kit as in claim 15, wherein the first container is a syringe.
17. A kit as in claim 16, wherein the second container is a syringe.
18. A kit as in any of the preceeding claims, wherein the aqueous re-hydration medium comprises thrombin.

19. A kit as in any of the preceeding claims, further comprising instructions for use setting forth a method for combining the cross-linked gelation powder and the re-hydration medium to produce a thrombin-containing fragmented gelatin hydrogel, and applying the hydrogel to a wound site.

20. A kit as in any of the preceeding claims, further comprising a package holding the first and second containers.

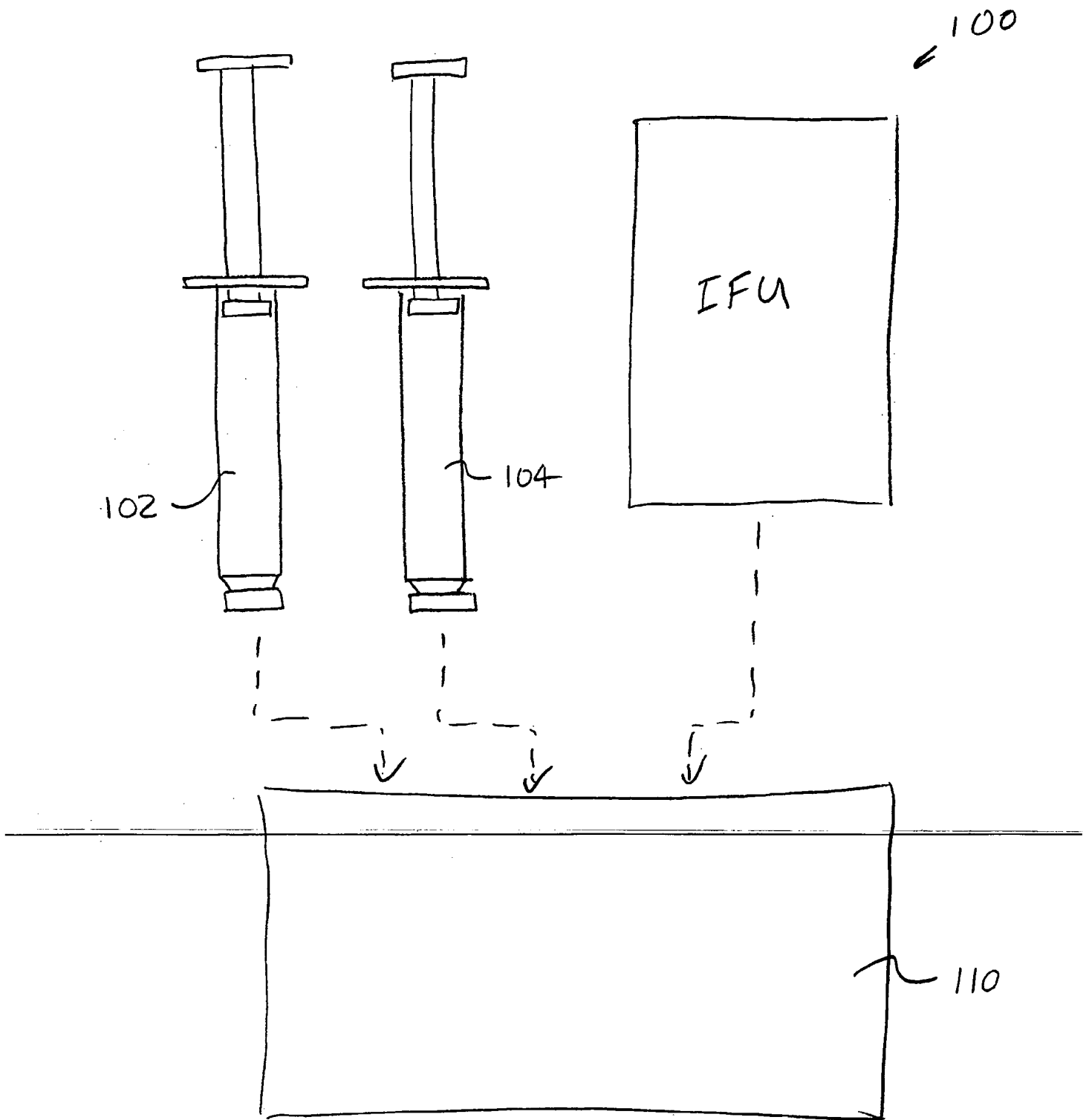


FIG - 1

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 2/02; A61K 38/39

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,543,332 A (JAO et al) 24 September 1985, see entire document	1-20

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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(54) Title: DRY HEMOSTATIC COMPOSITIONS AND METHODS FOR THEIR PREPARATION

(57) Abstract: Dry cross-linked gelatin compositions are prepared that rapidly re-hydrate to produce gelatin hydrogels suitable as hemostatic sealants. Gelatin is cross-linked in the presence of certain re-hydration aids, such as polyethylene glycol, polyvinyl-providone, and dextran, in order to produce a dry cross-linked gelatin powder. The use of the re-hydration aids has been found to substantially increase the re-hydration rate in the presence of an aqueous re-hydration medium, typically thrombin-containing saline.



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DRY HEMOSTATIC COMPOSITIONS AND METHODS FOR THEIR PREPARATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to collagen and collagen-derived compositions and methods for their preparation. In particular, the present invention relates to a method for producing a dry cross-linked gelatin or other collagen or collagen-derived composition which is capable of absorbing water at an enhanced rate.

Fusion Medical Technologies, Inc., assignee of the present application, produces a hemostatic composition under the FloSeal® trade name. The FloSeal® product is available in a package including two syringes. A first syringe is filled with granules of cross-linked bovine gelatin which are pre-hydrated with a buffer solution. The gelatin hydrogel contains about 85% (w/w) water and is in the form of a flowable hydrogel. Immediately prior to use in the operating room, thrombin in aqueous saline is mixed with the gelatin hydrogel. The thrombin is prepared in saline and drawn up in a second syringe, and the syringes are connected together permitting mixing of thrombin and the gelatin.

The resulting mixture of the gelatin hydrogel granules and the thrombin has been found to be a highly effective hemostatic sealant when applied to a bleeding site.

Typically, the sealant will be applied through the syringe in which it has been mixed to the bleeding site. Blood will percolate through the resulting bed of hydrogel granules, and the thrombin reacts with fibrinogen in the blood to form a fibrin clot around the gelatin to seal the bleeding site.

Although highly effective, the present FloSeal® product has a limited shelf life. It is believed that the stability of the gelatin is reduced by hydrolysis of the packaged hydrogel. To limit possible hydrolytic degradation, the FloSeal® product is usually shipped in a temperature-protected packaging.

For these reasons, it would be desirable to provide improved hemostatic sealing compositions of the type which combine a collagen, gelatin, or other collagen-derived hydrogel with a thrombin-containing aqueous solution. In particular, it would be desirable to provide such compositions in a form which would be resistant to hydrolytic degradation and which would therefore have a longer shelf life. It would be particularly desirable to provide

improved compositions having both comparable hemostatic activity to the present FloSeal[®] product and longer shelf lives. Such compositions would be most beneficial if they could be rapidly re-hydrated for subsequent use, typically so that they could be extruded through a syringe. At least some of these objectives will be met by the inventions described

hereinafter.

2. Description of the Background Art

The FloSeal[®] product available from Fusion Medical Technologies, Inc., is described in Hood et al., *Efficacy of Topical Hemostat FloSeal[™] in Vascular Surgery*, an Abstract funded by Fusion Medical Technologies, Inc., which was publicly presented in September 1999. Patents covering the FloSeal[®] product include U.S. Patent Nos. 6,063,061 and 6,066,325. A dual syringe system suitable for mixing and delivering a collagen, gelatin, or other collagen-derived component and a thrombin component of the FloSeal[™] product is described in U.S. Patent No. 5,908,054. The complete disclosures of each of these patent references is hereby incorporated by reference.

BRIEF SUMMARY OF THE INVENTION

The present invention provides improved hemostatic sealing compositions, methods for preparing such improved compositions, and kits comprising the improved compositions. The methods and compositions will be particularly useful for providing hemostasis at bleeding sites, including surgical bleeding sites, traumatic bleeding sites and the like. An exemplary use of the compositions may be in sealing the tissue tract above a blood vessel penetration created for vascular catheterization.

The compositions comprise a dry cross-linked gelatin powder which has been prepared to re-hydrate rapidly. The gelatin powder preferably comprises relatively large particles, also referred to as fragments or sub-units, as described in U.S. Patent Nos. 6,063,061 and 6,066,325, the full disclosures of which have previously been incorporated by reference. A preferred particle size will be the range from 150 μm to 750 μm , but particle sizes outside of this preferred range may find use in many circumstances. The dry compositions will also display a significant "equilibrium swell" when exposed to an aqueous re-hydrating medium. Preferably, the swell will be in the range from 400% to 1000%, but may fall outside of this range as set forth in the above-referenced patents. "Equilibrium swell" may be determined by subtracting the dry weight of the gelatin hydrogel powder from its weight when fully hydrated and thus fully swelled. The difference is then divided by the dry weight and multiplied by 100 to give the measure of swelling. The dry

weight should be measured after exposure of the material to an elevated temperature for a time sufficient to remove substantially all residual moisture, e.g., two hours at 120°C. The equilibrium hydration of the material can be achieved by immersing the dry material in a suitable re-hydrating medium, such as aqueous saline, for a time period sufficient for the water content to become constant, typically for from 18 to 24 hours at room temperature.

The dry cross-linked gelatin powders of present invention will usually have some residual moisture, but will be sufficiently dry to achieve the desired stability and extended shelf life. Typically, the dry compositions will have a moisture content below 20% by weight (w/w) or less, preferably having a moisture content in the range from 5% by weight to 15% by weight. To maintain dryness, the compositions will typically be packaged in a manner suitable to prevent moisture incursion, as described in more detail in connection with the kits of the present invention.

In one particular aspect of the present invention, compositions will comprise cross-linked gelatin powders having a moisture content of 20% (w/w) or less, wherein the powder was cross-linked in the presence of a re-hydration aid so that the powder has an aqueous re-hydration rate which is at least 5% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid. The "re-hydration rate" is defined herein to mean the quantity of an aqueous solution, typically 0.9% (w/w) saline, that is absorbed by a gram of the powder (dry weight basis) within thirty seconds, expressed as gm/gm. Particular techniques for measuring this rate are described in the Experimental section hereinafter. Preferred compositions of the present invention will have a re-hydration rate of at least 3 gm/gm, preferably at least 3.5 gm/gm, and often 3.75 gm/gm or higher. Re-hydration rates of similar powders prepared without the re-hydration aids are typically below three, and a percentage increase in re-hydration rate will usually be at least 5%, preferably being at least 10%, and more preferably being at least 25% or higher.

The dry cross-linked gelatin powders of the present invention having improved re-hydration rates are preferably obtained by preparing the powders in the presence of certain re-hydration aids. Such re-hydration aids will be present during the preparation of the powders, but will usually be removed from the final products. For example, re-hydration aids which are present at about 20% of the total solids content, will typically be reduced to below 1% in the final product, often below 0.5% by weight. Exemplary re-hydration aids include polyethylene glycol (PEG), preferably having a molecular weight of about 1000; polyvinylpyrrolidone (PVP), preferably having an average molecular weight of about 50,000; and dextran, typically having an average molecular weight of about 40,000. It is preferred to

employ at least two of these re-hydration aids when preparing the compositions of the present invention, and more particularly preferred to employ all three.

The methods of the present invention thus comprise providing an aqueous solution of a non-cross-linked gelatin combined with a re-hydration aid. The non-cross-linked gelatin will typically be present in an aqueous solution at from 5% (w/w) to 15% (w/w) and the re-hydration aids will be typically present from 5% to 30% (w/w) based on the weight of gelatin in the aqueous solution. Preferably, the re-hydration aid comprises PEG at from 2.5% to 20% (w/w) based on the weight of the gelatin, PVP at from 1.25% to 20% (w/w), and dextran at from 1.25% to 20% (w/w).

The non-cross-linked gelatin together with the re-hydration aid is then cross-linked in any manner suitable to form the hydrogel. For example, polymeric molecules may be cross-linked using bi- or poly-functional cross-linking agents which covalently attach to two or more polymer molecules chains. Exemplary bifunctional cross-linking agents include aldehydes, epoxies, succinimides, carbodiimides, maleimides, azides, carbonates, isocyanates, divinyl sulfone, alcohols, amines, imidates, anhydrides, halides, silanes, diazoacetate, aziridines, and the like. Alternatively, cross-linking may be achieved by using oxidizers and other agents, such as periodates, which activate side-chains or moieties on the polymer so that they may react with other side-chains or moieties to form the cross-linking bonds. An additional method of cross-linking comprises exposing the polymers to radiation, such as gamma radiation, to activate the polymer chains to permit cross-linking reactions. Dehydrothermal cross-linking methods may also be suitable. Preferred methods for cross-linking gelatin molecules are described below.

Exemplary methods for producing cross-linked gelatins are as follows.

Gelatin is obtained and suspended in an aqueous solution to form a non-cross-linked hydrogel, typically having a solids content from 1% to 70% by weight, usually from 3% to 10% by weight. The gelatin is cross-linked, typically by exposure to either glutaraldehyde (e.g., 0.01% to 0.05% w/w, overnight at 0 C to 15 C in aqueous buffer), sodium periodate (e.g., 0.05 M, held at 0°C to 15°C for 48 hours) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ("EDC") (e.g., 0.5% to 1.5% w/w overnight at room temperature), or by exposure to about 0.3 to 3 megarads of gamma or electron beam radiation. Alternatively, gelatin particles can be suspended in an alcohol, preferably methyl alcohol or ethyl alcohol, at a solids content of 1% to 70% by weight, usually 3% to 10% by weight, and cross-linked by exposure to a cross-linking agent, typically glutaraldehyde (e.g., 0.01% to 0.1% w/w, overnight at room temperature). In the case of aldehydes, the pH should be held from about 6

to 11, preferably from 7 to 10. When cross-linking with glutaraldehyde, the cross-links are formed via Schiff bases which may be stabilized by subsequent reduction, e.g., by treatment with sodium borohydride. After cross-linking, the resulting granules may be washed in water and optionally rinsed in an alcohol, and dried. The resulting dry powders may then be loaded
5 into the applicators of the present invention, as described in more detail hereinafter.

After cross-linking, at least 50% (w/w) of the re-hydration aid will be removed from the resulting hydrogel. Usually, the re-hydration aid is removed by filtration of the hydrogel followed by washing of the resulting filter cake. Such filtration/washing steps can be repeated one or more additional times in order to clean the product to a desired level and
10 to remove at least 50% of the re-hydration aid, preferably removing at least 90% (w/w) of the re-hydration aid originally present.

After filtration, the gelatin is dried, typically by drying the final filter cake which was produced. The dried filter cake may then be broken up or ground to produce the cross-linked powder having a particle size in the desired ranges set forth above.

15 Kits according to the present invention will comprise a first container holding the dry cross-linked gelatin powder of the present invention, as described above. The kits will further comprise a second container holding an aqueous re-hydration medium, typically a saline or other aqueous solution comprising thrombin which is intended to be mixed with the gelatin as the gelatin is re-hydrated. The containers can be in any form, but will preferably be
20 in the form of syringes which permit mixing of the dry gelatin with the re-hydration medium.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a kit constructed in accordance with the principles of the present invention.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

25 The following examples are offered by way of illustration, not by way of limitation.

Example 1: Preparation of gelatin powder

Strips of bovine corium were suspended in a sodium hydroxide solution of concentration 1 M to 2 M for 1 hr at room temperature, neutralized with phosphoric acid, and
30 rinsed. The treated strips were then resuspended in deionized water, adjusted to pH 7-8, and heated to 70°C. A homogenizer was used to further reduce the size of the strips. After 1 hr at 70°C, the corium was largely solubilized to gelatin. The amount of corium was chosen so

that the solids content of the resulting gelatin solution was approximately 3-10% (w/w), typically 7-10%. The solution was cast as thin layers onto Teflon® coated metal trays, dried, and ground to form gelatin powder.

Example 2: Preparation of “modified gelatin powder”

5 Re-hydration aids (Table 1) were dissolved in 500 mL of 50°C de-ionized water and then an amount of bovine derived gelatin powder, prepared as in Example 1, was added to the solution. The final concentration of gelatin in solution was chosen to be approximately 8% (w/w, bulk gelatin powder basis), and the total amount of re-hydration aids in the solution was chosen as in Examples 9-44 (Tables 1 and 2). After the gelatin had
10 dissolved, the solution was poured into Teflon® coated metal trays and dried. The dried gelatin sheet is ground to form “modified gelatin powder”.

Alternatively, strips of bovine corium were suspended in a sodium hydroxide solution of concentration 1 M to 2 M for 1 hr at room temperature, neutralized with phosphoric acid, and rinsed. The treated strips were then resuspended in deionized water,
15 adjusted to pH 7-8, and heated to 70°C. A homogenizer was used to further reduce the size of the strips. After 1 hr at 70°C, the corium was largely solubilized to gelatin. The amount of corium was chosen so that the solids content of the resulting gelatin solution was approximately 3-10% (w/w), typically 7-10%. Amounts of re-hydration aids were chosen as in Examples 9-44 (Tables 1 and 2) and were then added to the gelatin solution, either in solid
20 form or dissolved in a small volume of water. The solution was cast into thin layers onto Teflon® coated metal trays, dried, and ground to form “modified gelatin powder”. Examples of several formulations for modified gelatin are given in Tables 1 and 2.

Example 3: Preparation of cross-linked gelatin powder from “modified gelatin powder”

25 600 mL of 0.2 M phosphate buffer (pH 9.2 ± 0.2) was cooled to a temperature below 12°C. 0.32 mL of glutaraldehyde (25%) was added to the buffer solution and then 20 g of modified gelatin powder was added, resulting in a glutaraldehyde concentration of 4000 ppm (glutaraldehyde to modified gelatin, bulk weight basis). The gelatin was suspended in the glutaraldehyde solution with a stir bar. The pH of each suspensions was adjusted to a
30 range of 9.2 ± 0.2 and then maintained at a temperature of 9 to 12°C and pH of 9.2 ± 0.2 over 19 hours.

The suspension was filtered and the filter cake was washed with de-ionized water three times by completely covering the filter cake with de-ionized water and then

allowing the vacuum to draw the rinse water through the cake. The filter cake was left in the funnel during each rinse.

0.2 g of NaBH₄ was dissolved in 600 mL 25 mM phosphate buffer, pH 7.4
0.2, in a beaker. The above filter cake was suspended in the NaBH₄ solution at room
5 temperature (about 22°C) for 3 hours, then filtered to remove the liquid.

The filter cake was next suspended in 600 mL of buffer solution at room
temperature (about 22°C) for 30 minutes and filtered again. The buffer was composed of
sodium phosphate (dibasic anhydrous and monobasic monohydrate) and sodium ascorbate.
The above procedure was repeated twice to ensure that the appropriate ratio of salts to gelatin
10 were present to form the desired buffer composition upon reconstitution. The filter cake was
dried, then ground with a Waring Blender, resulting in "cross-linked gelatin powder".

This method was also used to prepare cross-linked gelatin powder from
unmodified gelatin powder; that is, gelatin to which no re-hydration aids were added during
its preparation.

15 Example 4: Preparation of irradiated product from cross-linked gelatin
powder

About 800 mg (bulk weight) of the cross-linked gelatin powder, prepared as in
Example 2, were put into each of several 5 cc syringes. The syringes containing powder
were sterilized with gamma irradiation at ambient temperature.

20 Example 5: Use of product as a hemostatic agent

A syringe of product containing approximately 0.8 g of irradiated cross-linked
gelatin powder was prepared from modified gelatin powder. The modified gelatin powder
was prepared as in Example 2. The modified gelatin was further cross-linked and irradiated
as in Examples 3 and 4. The product was mixed with 4 mL of a saline solution containing
25 about 1000 Units of bovine thrombin per milliliter. Mixing was achieved by passage back
and forth between two syringes connected with a female-female straight-through Luer
connector. The powder in the syringe was hydrated as it mixed with the thrombin solution,
forming granules of hydrogel.

A square lesion, approximately 1 cm x 1 cm x 0.2 cm deep, was created on the
30 liver of a farm-grade pig. The pig had been anticoagulated with heparin so that its activated
clotting time (ACT) was three to five times its baseline value, and the lesion bled freely prior
to treatment. After about 30 seconds from the start of mixing, approximately 2 mL of the
hydrated powder was extruded from the syringe onto the lesion and held in place with
compression for two minutes. After compression was removed, the treated lesion was

observed for bleeding at 3 min, 10 min, and 50 min after application. No bleeding was seen from the treated lesion at the 3 min and 10 min observation. After the 10 min observation, the treated lesion was irrigated with saline solution. While excess material was removed, no re-bleeding was observed. At 50 min after application, the lesion was observed again and no bleeding was seen.

Example 6: Determination of re-hydration rate of a powder

The “re-hydration rate” of a powder was measured as follows. The powder, packed in a 5 cc syringe, was mixed with a syringe containing a volume of aqueous solution by passage between the two syringes connected with a Luer fitting for 30 seconds. The volume of aqueous solution was chosen to be in excess of what could be expected to be absorbed in 30 seconds. Typically, 0.8 g (bulk weight) of powder was mixed with 3 mL of 0.9% sodium chloride solution. The resulting mixture was then immediately filtered to remove any unabsorbed liquid. The wet filtered material was weighed, then dried in a 120°C oven for two hours and re-weighed. This measurement gave the total amount of water removed from the wet material and the weight of the dry powder. The amount of water that had been absorbed by the powder was then calculated after a small correction is made for the residual moisture that had been present in the powder originally. The “re-hydration rate” was given as the mass of saline solution absorbed per gram dry weight of powder in that 30 second interval.

In the calculation below, the fraction solids of the bulk powder (“S”) was measured independently by drying the bulk powder at 120°C for 2 hr and weighing the powder before and after drying. The value of S is given by the following:

$$S = \frac{\text{weight after drying at } 120^{\circ}\text{C, 2hr}}{\text{weight before drying}}$$

Re-hydration rate calculation:

A: initial weight of the pan and filter paper

B: weight of the pan, filter paper and hydrated powder

C: weight of the pan, filter paper and sample after drying in oven

S: fraction solids of the bulk powder originally in syringe

M: grams of saline absorbed per gram of powder (dry weight) during mixing (“absorption rate”)

$$M = \frac{(B - A) - (C - A) / S}{(C - A)}$$

Example 7: Re-hydration rate and physical property determination for several batches of powder product

Tables 1 and 2 depict the results of re-hydration rate measurements performed on one to for several batches of powder product (Examples 9-23). These were made using methods as per Examples 1, 2, 3, and 4. Except for Examples 9 and 17, these were prepared from modified gelatins that were made with various proportions of gelatin and the following re-hydration aids: polyethylene glycol (PEG), average molecular weight 1000; polyvinylpyrrolidone (PVP), "k-30" designation, corresponding to an average molecular weight of about 50,000; and dextran, average molecular weight 40,000.

It is seen that use of several different combinations of gelatin and re-hydration aids can result in a powder product that absorbs more aqueous saline solution in 30 seconds per gram of powder than powder product made from gelatin to which no re-hydration aids have been added. It is also seen that the combination of gelatin, PEG, PVP and dextran at a bulk weight ratio of 80:10:5:5 in the modified gelatin (Example 10) produces a powder product that absorbs about 33% more saline solution per gram in 30 seconds than powder product made from unmodified gelatin.

Table 1 also gives values for other physical properties determined for the powder product lots. "Percent solids" was determined by weighing the powder before and after drying at 120°C for two hours to drive off residual moisture. "DSC peak temperature" refers to the temperature at which a peak is exhibited in a thermogram of a differential scanning calorimetry measurement conducted from 1°C to 70°C. "Equilibrium swell" was determined by suspending the powder in an excess of saline solution for at least 18 hr at room temperature. The hydrated powder was weighed to determine its "equilibrium wet weight" and dried at 120°C for two hours and re-weighed to determine its "dry weight". Equilibrium swell is given as

$$\text{Equilibrium swell (\%)} = 100\% \times \frac{\text{equilibrium wet weight} - \text{dry weight}}{\text{dry weight}}$$

Values for "mean particle size" were measured by light scattering in a Coulter LS particle size analyzer.

From the data presented in Table 1, it appears that the appropriate use of re-hydration aids can change the re-hydration rate of the powder product without significantly changing other physical properties.

Example 8: Measurement of polyethylene glycol, polyvinylpyrrolidone, and dextran levels in modified gelatin powder and in cross-linked powder

Approximately 50 mg modified gelatin or 250 mg cross-linked irradiated powder product were suspended in 10 mL of deionized water and heated for 3 hr at 65°C.

5 The samples were then centrifuged at 15 minutes at 2000 rpm. The resulting supernatant was filtered through a 0.45 µm Gelman Acrodisc filter, the first mL being discarded. The resulting sample was then assayed by three different high performance liquid chromatography (HPLC) methods to quantitate the polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and dextran in the sample. For PEG, 100 µL of the sample was
10 injected onto a Waters Ultrahydrogel 120 column, 7.8 x 300 mm, with guard column and prefilter, using deionized water as the mobile phase. A refractive index detector was used to monitor the effluent. For PVP, 100 µL of the sample was injected onto a Phenomenex Kingsorb C18 5µm column, 4.6 x 150 mm, with guard column and prefilter, using a gradient of methanol and aqueous sodium phosphate as the mobile phase. An ultraviolet absorbance
15 detector was used to monitor the effluent. For dextran, 100 µL of the sample was injected onto a Waters Ultrahydrogel Linear column, 7.8 x 300 mm, with guard column and prefilter, using 0.1 M sodium phosphate, pH 7 and acetonitrile at a 90:10 ratio as the mobile phase. A refractive index detector was used to monitor the effluent. All columns were heated to 40°C for the analyses. The limit of quantitation was about 0.1% (w/w sample) for PEG and PVP,
20 0.2% (w/w sample) for dextran.

Modified gelatin was prepared as per Example 2. The modified gelatin was analyzed for PEG, PVP and dextran in the manner described above. Results indicated that PEG, PVP, and dextran were present at 16%, 8%, and 3% (w/w bulk) respectively. The modified gelatin was subsequently subjected to cross-linking, sodium borohydride treatment,
25 and rinsing as per Example 3 to form cross-linked modified gelatin powder. When this powder was analyzed for PEG, PVP, and dextran by HPLC in the manner described above, the content of each of the three re-hydration aids was found to be below the limit of quantitation.

Example 9: Powder product made without re-hydration aids

30 Unmodified gelatin—that is, gelatin to which processing aids were not added—was prepared from bovine corium strips as in Example 1 and cross-linked as in Example 3. The cross-linked unmodified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting product were measured as in Examples 6 and 7 and are given in Table 1.

Examples 10-23: Powder product made with re-hydration aids

Batches of modified gelatin were prepared as in Example 2 from gelatin powder or corium strips and from one, two, or three re-hydration aids. Table 1 gives the proportions of bulk gelatin and re-hydration aids used. The modified gelatin was then cross-linked as in Example 3. Except for Example 17, the re-hydration aids used were from the following list: polyethylene glycol (PEG) of an average molecular weight of about 1000; polyvinylpyrrolidone (PVP), "k-30" designation, of an average molecular weight of about 50,000; and dextran, of an average molecular weight of about 40,000. In Example 17, PEG of an average molecular weight of about 400 was used. The cross-linked modified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting powder product from each of these preparations were measured as in Examples 6 and 7 and are given in Table 1. Data given with the formulation for Example 10 is the average and standard deviation of nine batches prepared according to that formulation.

Examples 24-44: Powder product made with various re-hydration aids

Batches of modified gelatin were prepared as in Example 2 from gelatin powder or corium strips and from one of several re-hydration aids. Table 2 gives the identity and concentration of re-hydration aid used in each batch as a ratio of bulk gelatin weight to re-hydration aid and as a percentage of total bulk solute used to prepare the modified gelatin. The modified gelatin was then cross-linked as in Example 3. The cross-linked modified gelatin was then packed into syringes and gamma irradiated as in Example 4. Physical properties of the resulting powder product from each of these preparations were measured as in Examples 6 and 7 and are given in Table 2. Data for the Example 9 formulation is provided in Table 2 for comparison.

TABLE 1

		Target bulk weight percent in modified gelatin				Properties of powder product after cross-linking and gamma irradiation (re-hydration aids largely removed)				
	Lot	Gelatin (bulk weight)	PEG MW = 1000 D	PVP MW ~ 50000 D	Dextran MW = 40000 D	% solids	DSC peak temp (°C)	Equilibrium swell (%)	Mean particle size (µm)	Re-hydration*
No re-hydration aids added										
Example 9	208-32	100	0	0	0	88.6	41.3	551	440	2.85
Preferred composition (four-way mixture)										
Example 10	avg of 9 lots	80	10	5	5	87.6	42.1	595	423	3.79
	std. deviation of 9 lots					1.0	1.4	43	65	0.15
Three-way mixtures										
Example 11	228-69-1	80	10	10	0	88.1	40.8	667	387	3.51
Example 12	228-69-2	80	10	0	10	88.4	40.6	670	367	3.14
Example 13	228-78	80	0	10	10	86.7	41.1	632	414	3.20
Gelatin-PEG mixtures										
Example 14	212-39-2	94	6	0	0	86.2	44.4	502	372	2.68
Example 15	228-42-3	89	11	0	0	88.6	42.8	594	428	3.16
Example 16	228-42-1	80	20	0	0	88.9	42.4	575	312	3.47
Example 17	214-62-1	89	11**	0	0	87.1	40.7	599	406	3.11
Gelatin-PVP mixtures										
Example 18	228-38-3	94	0	6	0	88.2	42.2	567	399	3.26
Example 19	228-38-2	89	0	11	0	88.3	41.0	605	422	3.44
Example 20	228-38-1	80	0	20	0	88.6	42.4	596	401	3.52
Gelatin-dextran mixtures										
Example 21	228-35-3	94	0	0	6	88.1	40.5	631	395	3.18
Example 22	228-35-2	89	0	0	11	88.3	41.4	598	345	3.03
Example 23	228-35-1	80	0	0	20	88.5	41.9	624	392	3.01
* Re-hydration rate defined as grams saline absorbed per gram powder product (dry wt) in 30 sec										
** PEG (MW =400) used instead of MW=1000										

TABLE 2

	Lot	Re-hydration aid			Conc'n of processing aid in modified gelatin (bulk wt%)	Physical properties				
		type	MW or other designation	bulk gelatin wt: aid		% solids	DSC peak temp (°C)	Equilibrium swell (%)	Mean particle size (µm)	Re-hydration rate*
Example 9	208-32	no re-hydration aids				88.6	41.3	551	440	2.85
Example 24	214-11-1	glycerol	n/a	4	20%	85.5	43.4	483	653	2.19
Example 25	214-11-2	glycerol	n/a	8	11%	86.4	43.4	529	421	2.62
Example 26	214-11-3	glycerol	n/a	16	6%	86.5	43.0	543	398	2.35
Example 27	214-44-1	dextran	148000 D	4	20%	85.5	nr	634	433	2.62
Example 28	214-44-2	dextran	148000 D	8	11%	85.4	nr	607	453	2.57
Example 29	214-44-3	dextran	148000 D	16	6%	85.5	nr	603	527	2.33
Example 30	214-44-4	dextran	148000 D	32	3%	85.7	nr	531	491	2.37
Example 31	228-35-4	dextran	40000 D	32	3%	84.5	41.4	633	380	2.59
Example 32	214-50-1	PVP	k-90	4	20%	85.3	44.0	612	664	2.41
Example 33	214-50-2	PVP	k-90	8	11%	85.6	44.3	538	581	2.71
Example 34	214-50-3	PVP	k-90	16	6%	85.6	44.1	527	593	2.78
Example 35	214-50-4	PVP	k-90	32	3%	86.1	43.0	597	538	2.76
Example 36	214-53-4	PVP	k-30	32	3%	87.3	41.1	580	447	2.72
Example 37	214-59-1	PEG	400	4	20%	86.7	42.0	595	407	2.18
Example 38	214-66-1	PEG	400	6	14%	86.5	40.8	603	501	2.63
Example 39	212-39-1	PEG	400	16	6%	86.2	43.8	513	403	2.11
Example 40	212-39-2	PEG	1000	16	6%	86.2	44.4	502	372	2.68
Example 41	214-59-3	PEG	8000	4	20%	87.4	41.5	548	429	2.87
Example 42	214-66-3	PEG	8000	6	14%	86.9	41.4	581	426	3.80
Example 43	214-62-3	PEG	8000	8	11%	86.8	42.0	631	511	2.78
Example 44	212-39-3	PEG	8000	16	6%	86.4	44.6	546	518	2.72

nr = not reported *-Re-hydration rate defined as grams saline absorbed per gram powder product (dry wt) in 30 sec

While the above is a complete description of the preferred embodiments of the invention, various alternatives, modifications, and equivalents may be used. Therefore, the above description should not be taken as limiting the scope of the invention which is defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing a substantially dry cross-linked gelatin powder, said method comprising:
 - providing an aqueous solution comprising linked gelatin combined with at least one re-hydration aid;
 - drying the solution of the gelatin and re-hydration aid to produce solids;
 - grinding the solids to produce a powder;
 - cross-linking the powder;
 - removing at least 50% (w/w) of the re-hydration aid; and
 - drying the cross-linked gelatin to produce a powder having a moisture content below 20% (w/w).
2. A method as in claim 1, wherein the re-hydration aid comprises one, two, or three of the materials selected from the group consisting of polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and dextran.
3. A method as in claims 1 or 2, wherein the re-hydration aid is present at a concentration in the range from 5% to 30% by weight based on the weight of gelatin present in the aqueous solution.
4. A method as in claim 3, wherein the re-hydration aid comprises PEG at from 2.5% to 20% by weight, PVP at from 1.25% to 20% by weight, and dextran at from 1.25% to 20% by weight.
5. A method as in any of the preceeding claims, wherein cross-linking comprises adding a cross-linking agent to the gelatin solution.
6. A method as in claim 5, wherein the cross-linking agent comprises glutaraldehyde.
7. A method as in any of the preceeding claims, wherein removing at least 50% of the re-hydration aid comprises filtering the suspension of cross-linked powder in a solvent followed by filtration gelatin to produce a filter cake and washing the filter cake to remove the re-hydration aid.

8. A method as in claim 7, wherein washing the filter cake removes at least 90% (w/w) of the re-hydration aid originally present in the gelatin.
9. A method as in any of the preceeding claims, wherein drying comprises drying the filter cake after washing, wherein the method further comprises drying the ground filter cake to produce the dry ground gelatin powder.
10. A composition comprising cross-linked gelatin powder having a moisture content of 20% (w/w) or less, wherein the powder was cross-linked in the presence of a re-hydration aid so that the powder has an aqueous re-hydration rate which is at least 5% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid.
11. A composition as in claim 10, wherein the powder has a re-hydration rate which is at least 10% higher and optionally at least 25% higher than the re-hydration rate of a similar powder prepared without the re-hydration aid.
12. A composition as in claims 10 or 11, wherein the re-hydration aid comprises one, two, or three of the materials selected from the group consisting of polyethylene glycol (PEG) polyvinylpyrrolidone (PVP), and dextran.
13. A composition as in any of the preceeding claims, wherein the powder has a mean particle size in the range from 150 μm to 750 μm .
14. A composition as in any of the preceeding claims, wherein the powder has an equilibrium swell in the range from 400% to 1000%.
15. A kit comprising:
 - a first container holding cross-linked gelatin powder any of claims 12-19; and
 - a second container holding an aqueous re-hydration medium.
16. A kit as in claim 15, wherein the first container is a syringe.
17. A kit as in claim 16, wherein the second container is a syringe.
18. A kit as in any of the preceeding claims, wherein the aqueous re-hydration medium comprises thrombin.

19. A kit as in any of the preceeding claims, further comprising instructions for use setting forth a method for combining the cross-linked gelation powder and the re-hydration medium to produce a thrombin-containing fragmented gelatin hydrogel, and applying the hydrogel to a wound site.

20. A kit as in any of the preceeding claims, further comprising a package holding the first and second containers.

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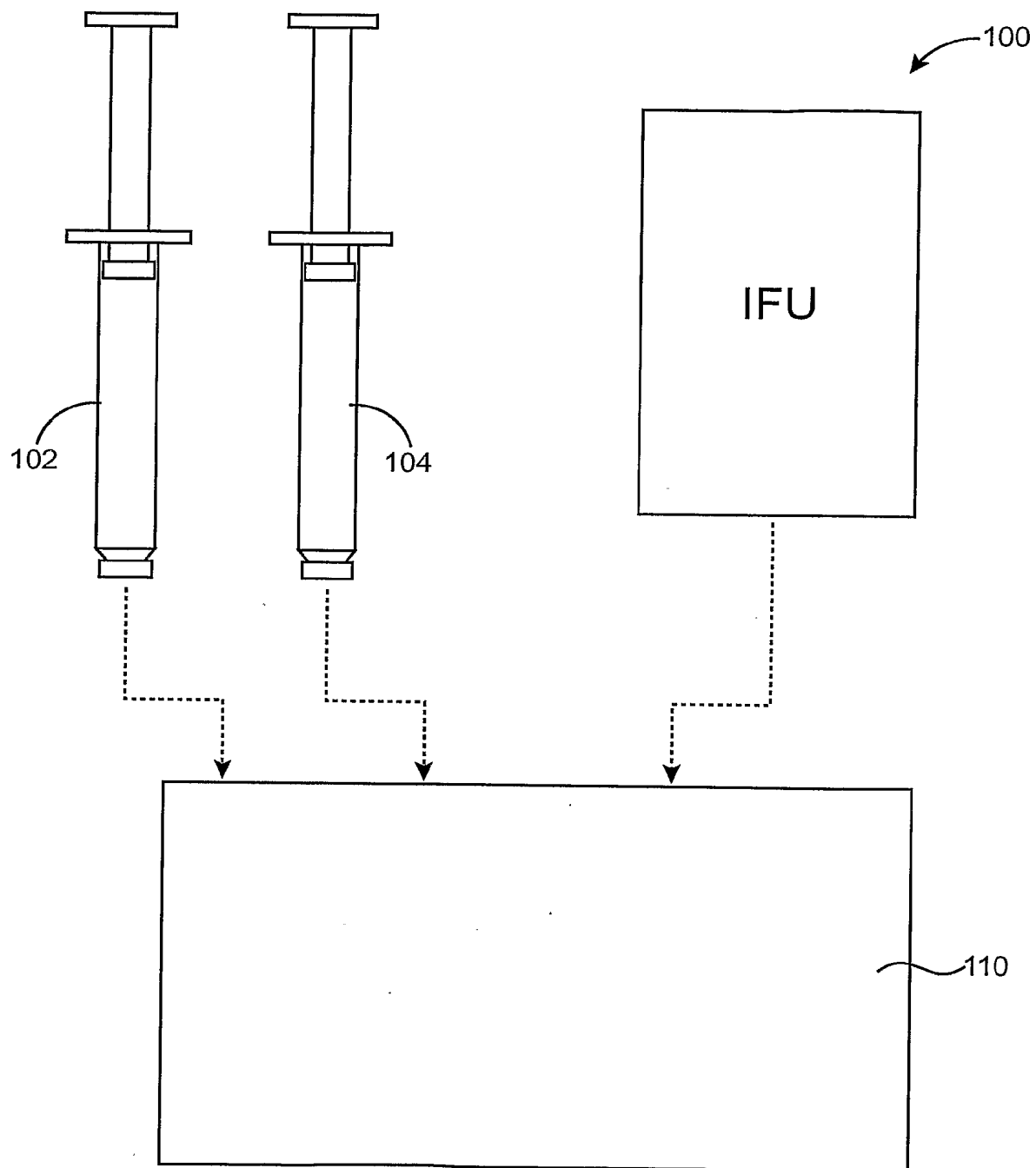


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/20022

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 2/02; A61K 38/39

US CL : 424/423, 426; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/423, 426; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,543,332 A (JAO et al) 24 September 1985, see entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

24 JULY 2002

Date of mailing of the international search report

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